

Comparing SYBR Green I dye and TaqMan Assays for real-time PCR analysis

Real-time PCR instruments detect fluorescent signals generated during target amplification. Thermo Fisher Scientific offers products for two distinct detection chemistries that are widely used in research for fluorescence-based analysis of nucleic acids by real-time PCR.

Reaction chemistry

SYBR Green I dye

vs.

TaqMan Assays

Dye-based detection: Invitrogen™ SYBR™ Green I dye binds nonspecifically to double-stranded DNA, then fluoresces upon excitation.



SYBR Green dye-based assays include a forward primer and a reverse primer designed to amplify a specific target sequence. The dye is added to the reaction separately, usually as a component of a master mix.



SYBR Green I dye produces a fluorescent signal whether bound to the amplified target sequence, nonspecific amplification products, or primer-dimers.



Suitable for applications with **low specificity** demands:

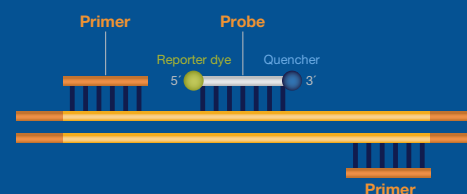
-  Mycoplasma infection in cell culture
-  Next-generation sequencing (NGS) library quantification
-  Telomere length
-  Chromatin immunoprecipitation (ChIP)

Detection method

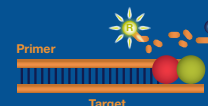
Probe-based detection: Sequence-specific Applied Biosystems™ TaqMan™ Assay probes are labeled with a fluorescent reporter dye and a quencher.



TaqMan Assays for gene expression analysis include two PCR primers and a gene-specific TaqMan probe.



During polymerization, the 5' nuclease activity of *Taq* DNA polymerase cleaves the bound probe. Once separated from the quencher, the reporter dye emits a permanent fluorescent signal.



Ideal for applications with **high specificity** demands:

-  Gene expression analysis
-  miRNA analysis
-  Pathogen detection or quantification
-  Copy number variation, SNP genotyping
-  Clinical research

When to use

Assay specificity

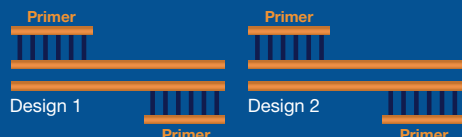
The specificity of an assay is its ability to detect targets and exclude nontargets in samples. The use of 3 oligonucleotides in TaqMan Assays helps to ensure **higher specificity** than the 2 oligonucleotides used in SYBR Green dye-based assays.

SYBR Green I dye

vs.

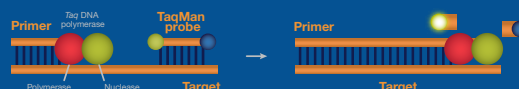
TaqMan Assays

Target detection relies on 2 primers, which must be optimized for specificity.



Ability to detect target

Target detection relies on 2 primers **and** cleavage of the bound sequence-specific probe, helping ensure higher specificity.



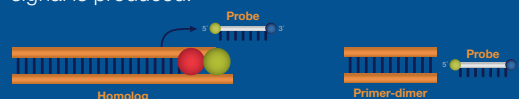
Signals from nontargets cannot be excluded. Post-run specificity monitoring is recommended, but because of inherent limitations it does not substitute for specificity verification.

- Melt curve analysis
- Gel electrophoresis
- Sequencing

Ability to exclude nontargets

TaqMan probes help ensure nontargets are excluded. Mismatched probes are displaced from homologs without being cleaved, so no signal is produced.

TaqMan probes cannot bind to nonspecific PCR products (e.g., primer-dimers) and become cleaved, so no signal is produced.



Workflow design and optimization

SYBR Green I dye

vs.

TaqMan Assays

Predefined assays are not available for gene expression analysis using SYBR Green I dye. Developing an assay typically requires user design and experimental optimization.

Assay design and optimization

Thermo Fisher Scientific offers over 2.8 million verified, ready-to-use **TaqMan Gene Expression Assays** for research, with guaranteed* performance.

* Terms and conditions apply. To see full details of the guarantee, go to thermofisher.com/taqmanguarantee.

No

Multiplexing

Yes

Applied Biosystems™ PowerTrack™ SYBR™ Green Master Mix

Recommended reagents

Applied Biosystems™ TaqMan™ Fast Advanced Master Mix or **TaqMan™ Fast Virus 1-Step Master Mix**

Learn more about real-time PCR products and applications at thermofisher.com/qpcr

applied biosystems